UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Investigating the applicability of the CEGAP model to predict the development of harmful algae blooms in the Klipvoor Dam

By

Innocent Sazi Mthembu

A minor dissertation submitted in partial fulfillment of the requirements of degree

MASTER OF SCIENCE WATER RESOURCE MANAGEMENT

In the

Faculty of Natural and Agricultural Sciences

University of Pretoria

Supervisor: Dr C.E. Van Ginkel

August 2016

DECLARATION

I, Innocent Sazi Mthembu, declare that the dissertation, which I hereby submit for the degree of Master of Science Water Resource Management at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Sazi Innocent Mthembu (Mr)	Date

Investigating the applicability of the CEGAP model to predict the development of harmful algae blooms in the Klipvoor Dam

Student:	Innocent Sazi Mthembu
Supervisor:	Dr C.E. Van Ginkel
Department:	Centre for Environmental Studies (CFES)
Faculty:	Faculty of Natural and Agricultural Sciences
University:	University of Pretoria Hatfield, South Africa 0028
Degree:	Master of Science in Water Resource Management

Abstract

South Africa is a water scarce country depending primarily on reservoirs and lakes for socio-economic wellbeing. Most of these reservoirs are contaminated with nutrients making them either eutrophic or even hypertrophic. The algae blooms are common amongst these reservoirs. There is however a worrying trend of an increasing number of reservoirs with cyanobacterial blooms. Cyanobacterial blooms produce cyanotoxins which may result in human and animal deaths. Therefore, it is important to have a reliable tool that can predict emergence of these blooms for the South African reservoirs. The CEGAP model has been developed for hypertrophic reservoirs of South African to predict the emergence of *Microcystis spp*. blooms. The objective of this research was to test the applicability of the CEGAP model at Klipvoor Dam. Ten year data from the Department of Water and Sanitation database was used to assess the applicability and accuracy of the CEGAP model to predict occurrence of the cyanobacterial blooms for the Klipvoor Dam.

The model was used to predict emergence of the *Microcystis spp*. blooms for real time, 14 days and 28 day forward predictions. The results show that the model was able to predict the emergence of the algae blooms for the real time and 14-day forward predictions. The model could not predict the emergence of blooms for 28-day forward predictions reliably. The model could predict the emergence of bloom events with reasonable accuracy but could not predict accurately the severity of the blooms.

The results indicate that the model is able to predict the emergence of the *Microcystis* biomass but the severity of the biomass may be influenced by other algae species, which in certain instances may be larger in size and extent than the *Microcystis* species, and hence contribute a larger percentage of the biomass and chlorophyll-*a* concentration, even if they are low in terms of the species counts.

It was therefore recommended that the models ability to predict the severity of the blooms and its ability to predict the emergence of other algae species blooms such as *Ceratium spp.* be investigated further.

ACKNOWLEDGEMENTS

First I would like to thank God the Almighty for life of abundance; to Him all praise is due. I would like to acknowledge the following people for their support:

- The Department of Water and Sanitation’s management for providing financial support for the study.
- My supervisor and mentor Dr. Carin Van Ginkel for her guidance and support during the project.
- My family (my wife Thobile and children Naledi, Lesedi and Mphumeleli) for their understanding and support during my studies.
- Resource Quality Information Services (RQIS): Laboratory Services staff for providing data, monitoring personnel for monitoring of sampling sites and WMS personnel for making data available when required.
- Resource Quality Management (RQM) staff for always providing guidance when required.
- Marinda Cilliers for her support and encouragement while doing the Water Resource Management degree.

TABLE OF CONTENTS

CHAPTER 1

INTRODUCTION

1.1 Background

The use of various models to simulate eutrophication in South Africa dates as far back as the mid-1980s (Grobler 1985, Rossouw 1990). The testing of a variety of models is one of the methods applied in water resource management in South Africa. In a study undertaken by Venter and Herold (2001) the applicability of the MINLAKE model was tested at Roodeplaat Dam. This is one of the key studies about the applicability of models in South African dams. MINLAKE model is a one-dimensional water quality model that was originally developed at the University of Minnesota in the USA. The model had an extensive application in the lakes and impoundments in the northern USA. The model has since been modified and tested for the applicability in the temperate to subtropical warmer climates of South Africa (Venter and Herold 2001). The modified MINTEK model could predict the change in the phosphorous concentration but failed to predict the degree of change in chlorophyll-*a*, ammonia and nitrate concentration. This led to significant decrease in the ability of the model to predict the future trophic conditions in impoundments. The inaccuracies in the MINTEK model to predict trophic status meant that models suitable to South African conditions had to be developed and validated. In the recent study by Van Ginkel (2008) an algorithm has been developed to predict the emergence of cyanobacteria in South African resources. This model is called the CEGAP model (Barnard et al. 2014). The model has been tested at Rietvlei Dam and Bronkhorstspruit Dam where it was able to predict blooms timely and with accuracy (Barnard et al. 2014). The model was demonstrated to be capable of predicting *Microcystis spp.* and *Ceratium spp.* for Rietvlei Dam and Bronkhorstspruit Dam, respectively (Barnard et al. 2014). The data from Klipvoor Dam was used in this research to investigate the applicability and accuracy of the CEGAP model in predicting the emergence of algal blooms in the dam.

1.2 Problem Statement

Klipvoor Dam is one of the hypertrophic reservoirs in South Africa and experiences algal blooms during the warmer months of the year. This often results in negative impacts such as fish kills and economic losses due to reduced recreational activities, when the blooms are present. It can also lead to animal deaths as the impoundment/dam is situated in a nature reserve. In the study undertaken by Masango for the wildlife mortality in the Kruger National park it was concluded that eutrophication and *Microcystis* bloom formation were the causes of the wildlife mortality, particularly hippopotami, during the 2007 period (Masango et al. 2010). In another study Masango found that animals such as zebra, rhino and wildebeest died from drinking *Microcystis aeruginosa* contaminated water and when the post mortem and histopathology were performed on the zebra carcasses it revealed signs (hepatic haemorrhaging and necrosis) that are consistent with *Microcystis* bloom intoxication (Masango et al. 2012). The predictions are that the incidences of animal death due to microcystin poisoning will increase and intensify in the future due to increasing temperatures as a result of climate change (Oberholster et al. 2009). It is therefore important to test and develop functional models that are applicable to the South African systems and conditions which can be used to predict emergence of *Microcystis* blooms for effective management of water resources.

1.3 Key Research Question

The main question to ask is whether the hybrid evolutionary algorithm (HEA) rules that have been developed for South African reservoirs, namely CEGAP model, may be used to predict the emergence of harmful algal blooms in Klipvoor Dam.

1.4 Hypothesis

South Africa shows an upward trend in cyanobacterial bloom incidences (Codd et al. 2005, Guven & Howard 2006, Matthews 2015). In the recent study undertaken by Matthews for the 2002 to 2012 period using observations from satellite imagery for 50 of the South Africa’s largest water bodies, it was found that 36 of them were hypertrophic (chl-*a* greater than 30mg/m^3), 3 were eutrophic (chl-*a* of 20–30mg/m^3), 4 were mesotrophic (chl-*a* of 10-20mg/m^3) and 7 were oligotrophic (chl-*a* less than 10mg/m^3). In the same study cyanobacterial blooms were found in all 50 of the water bodies studied (Matthew 2015). The eutrophied water systems have been noted to have instances of cyanobacteria blooms. The bloom formation is stimulated by elevated nutrients (P and N), water temperatures (15 – 30^{o}C) and a pH between 6 and 9 (Wicks and Thiel 2008).

There is potential for cytotoxic poisoning for the water users (including people who use the water for potable consumption, fishing and wildlife). One of the key uses of the dams is abstraction for potable treatment at water treatment plants. The treatment of the contaminated water tends to be more expensive compared to the normal unpolluted water. It is therefore imperative to develop a model that can be used by these water treatment plants to predict the occurrence of the potential for cyanotoxin occurrences during blooms. Such a model can provide early warning so that the necessary supplies may be purchased to treat the water to potable standards. There are various modeling techniques that have been tested to be able to predict the occurrence of these blooms. The challenge is that these models were not developed here in South Africa and they tend to often be incompatible with South African conditions. These include the Vollenweider Model, the SALMO Model, Artificial Neural Networks (ANN), and Hybrid Evolutionary Algorithms. The HEA rule has been developed previously and tested by Van Ginkel (2008), for the hypertrophic dams of South Africa. These were found to be applicable to the South African conditions. The objective in the current study is evaluating the applicability of this HEA rule in the Klipvoor Dam.

Hypothesis: The Hybrid Evolutionary Algorithm rules (specifically CEGAP model) set can be used with confidence to predict cyanobacterial blooms in the Klipvoor Dam system.

CHAPTER 2

LITERATURE REVIEW

2.1 Eutrophication

South Africa is a water scarce country relying heavily on reservoirs for assurance of supply to the communities. About 66% of the country's mean annual rainfall is stored in about 320 major reservoirs around the country (NWRS 2004). South Africa is an arid country with an average rainfall of 450mm per annum, which is about half of the world average of 860mm per annum. The high evaporation rate means that the available water is lost, which add to the water scarcity. The availability of the fresh surface water becomes critical in order to drive the economic and cultural activities to prosperity (Gophen 2008).

The excessive enrichment of water bodies with plant nutrients is one of the serious water quality problems around the world (Grobler 1986). Eutrophication has been defined to be the process by which a body of water acquires a high concentration of nutrients, especially phosphates and nitrates (Art 1993; Nixon 1995). These typically promote symptomatic changes in the lakes such as excessive growth of algae and aquatic macrophytes leading to the deterioration of water quality (OECD 1982). The excess algae eventually die off and decompose. This together with high levels of organic matter depletes the water of available oxygen, causing the death of other organisms, such as fish (Art 1993; Lawrence and Jackson 1998). In the event of a cyanobacterial bloom the decomposing bloom will also produce serious cyanotoxin events that can lead to human and animal deaths (Owour *et al*. 2007). Eutrophication is a natural, slow-aging process for a water body, but human activity (cultural eutrophication from fertilizer runoff and sewage discharge) greatly speeds up the process (Art 1993; Lawrence and Jackson 1998).

Eutrophication is influenced by natural processes such as the geology around the lake and it is part of the ageing process of the lakes (Wetzel 2003). The process, if left to occur naturally, may take tens of thousands of years to change the nature of the lake. Eutrophication becomes a problem as it is enhanced by human activities such as farming and industrial activities in the catchment and around the lakes.

2.2 Effects of Eutrophication

Eutrophication may lead to ecological changes in and around the lakes. It is reported that in lakes, such as Lake Victoria, ecological changes are seen and include increases in phytoplankton primary production (Hecky and Bugenyi 1992; Muggide 1993). Other changes that have been observed include (1) replacement of diatoms by cyanobacteria as the dominant group of planktonic algae (Kling et al. 2001), (2) blooms of aquatic weeds e.g. water-hyacinth, and (3) extinction of some endemic fish species (Barel et al. 1985) as they are replaced by species that can tolerate low oxygen levels.

Eutrophication that lead to rapid growth of phytoplankton species and macrophytes, often lead to mono-species blooms (Oberholster et al. 2009) of the cyanobacterial species, especially *Microcystis* and *Anabaena*, presence in South Africa. The blooms disrupt normal patterns of phytoplankton succession, decreases diversity and alter interaction between organisms within the aquatic community (Figueredo and Giani 2001). The consequence is the production of secondary metabolites that may have serious consequences to health and vitality of humans and animals (Wiegand and Pflugmacher 2005).

The accumulation and decomposition of dead organic matter consumes oxygen and generates harmful gases such as methane and hydrogen sulphide. This suffocates many species including macro-invertebrates and fish species, whilst the immobile bottom dwelling species may die off completely (Nyenje 2010).

2.3 Phytoplankton

Phytoplankton are photosynthetic, free-floating organisms found in lakes, slow-flowing rivers, estuaries and oceans, and they are, amongst others, the most common photosynthetic organisms on the planet (Horne & Goldman, 1994). The phytoplankton may affect features of the lake such as colour, clarity, trophic state, water chemistry, the taste and odour of water, zooplankton- and fish production. Surface waters may support growth of phytoplankton when the conditions such as temperature, light and nutrient availability are favourable (Swanepoel et al. 2008).

2.4 Cyanobacteria

Cyanobacteria are ancient organisms of which the blooms have been recorded through history (photosynthetic prokaryotes found mainly in illuminated environments). These photosynthetic prokaryotes possess both bacterial and algal characteristics (Chorus 2001). They synthesize chlorophyll *a*, and water usually acts as the electron donor during photosynthesis in the process that leads to the physiological evolution of oxygen. They are prokaryotes which lack a nuclear membrane, mitochondria and chloroplasts. The most toxic freshwater cyanobacteria are *Microcystis spp*., *Anabaena spp*., and *Planktothrix spp*., with the most common toxins produced being microcystins, especially microcystin-LR (Hitzfeld et al. 2000)

Most of these species produce phycobilin pigment, phycocyanin, which gives the cells a bluish colour when present in sufficient quantities. These are responsible for the popular name blue-green algae, in some instances a red pigment called phycoerythrin may be produced. The cyanobacteria may be found on moist soils, footpaths, tidal cliffs and sidewalks kept wet by moist environments (Whitton & Potts 2000). They are both unicelluar and filamentous organisms.

The temperature optimum for many cyanobacteria is higher by several degrees than for most eukaryotic algae (Castenholtz and Waterbury 1989) thus encouraging their success in warmer climates (Koster et al. 2012). The predicted impact of climate change is that temperatures will increase by a few degrees in the near future (Clarke 2006), these increases may lead to increased incidences of cyanobacteria blooms as these species thrive in higher temperatures. They can tolerate free sulphides at much higher levels than most eukaryotic algae and may use H_2S as electron donor during photosynthesis (Cohen et al. 1986). They can continue with photosynthetic CO_2 reduction even at very low inorganic carbon concentrations (Pierce and Omata 1988). They can also form gas vacuoles which increases cell buoyancy and hence can move around the water column even when there is low vertical mixing. This phenomenon is not found in all the cyanobacterial species.

Microcystins are chemically stable compounds so that conventional water treatment methods such as flocculation, sedimentation, rapid sand filtration and chlorination have limited efficacy in removing the toxins to WHO accepted concentrations

(Izaguirre et al. 1982; Cornish et al. 2000; Liu et al. 2002, 2003, 2009; Schijven et al. 2003; Oberholster et al. 2005; Valeria et al. 2006; Swanepoel et al. 2008). The toxins gets released by treatment processes that utilise chemicals such as potassium permanganate or chlorine and the toxins can eventually reach the people through potable water supplies (Chow et al. 1998; Peterson et al. 1995; Van Apeldoorn et al. 2007).

The advanced treatment processes like granular activated carbon filtration are, however, able to remove these cyanobacterial toxins from water (Oberholster et al. 2005). In South Africa only few water treatment works are equipped with an advanced treatment process which includes granular activated carbon filtration. The conventional methods of purification only remove cyanobacterial cells but not the biotoxins from water (Oberholster et al. 2005), as it only breaks down the cyanobacterial cells that lead to biotoxin release into the water which moves through the purification works.

Cyanobacteria are able to store nutrients i.e. nitrogen and phosphorous when nutrients and other growth requirements are limiting. This process is called luxury uptake. These excess nutrients are later used by the cells to fuel growth when there is limited supply of nutrients. Nutrient limitation of cyanobacteria may cause either general stress responses as a result of detained anabolism or specific responses such as acclimation processes specific to a particular nutrient. This leads to the modification of metabolic and physiological activities in order to compensate for the limitations (Schwarz and Forchhammer 2005).

The general response of phytoplankton to nutrient limitation include: carbohydrate accumulation, a reduction in the cell-specific quantum yield of photosynthesis (Turpin 1991), a reduction in the cellular content of the limiting nutrient (Riegman and Mur 1984) and an increase in the specific (luxury) uptake of the limiting nutrient (Riegman and Mur 1984; Kromkamp 1987). The limitation of nutrients stimulates storage of non-limiting nutrients as a result of their relative excess compared to the diminished requirement for the cell (Whitton BA 2012). The process of nutrient storage is important as it allows the cell to use pools of nutrients which may be spatially and temporally separated in order to maintain cell growth during periods of nutrient scarcity (Whitton BA 2012).

2.5 Phosphorous

In phosphorous limiting conditions cellular phosphorous concentrations decline as the phosphorous limited growth rate declines, while the uptake potential increases. As a consequence, a pulse of phosphorous delivered to phosphorous-limited cells results in substantial formation of polyphosphate reserves. The polyphosphate oversupply phenomenon, with cellular phosphorous levels is able to exceed those under steady state maximum growth rates (Allen 1984; Riegman and Mur 1984). Most of the phytoplankton species can store surplus phosphorous, usually in the form of polyphosphate and these may be enough to support several cell doublings.

2.6 Nitrogen storage

Cyanobacteria have the potential to store significant amounts of nitrogen in excess of their immediate requirements. Nitrogen is stored either as phycocyanin or cyanophycin (Kolodny et al. 2006).

Many of the lake studies have focused on total nutrients in the lake rather than the bioavailable forms. The inorganic form is generally considered to be the bioavailable form but the organic form can be utilised as the important component of the nutrient uptake particularly in the presence of phosphatise to mobilise organic phosphorous (Berman 1997). The lakes in the past have been analysed on the basis of the Redfield ratio, where it was suggested that cyanobacteria should dominate in the waters with low N:P ratios (Downing et al. 2001). Recent work, however, has since questioned this conclusion in that the individual nutrient concentration of either total phosphorous or total nitrogen seem to provide a more reliable estimation of average cyanobacterial dominance than their ratios (Trimbee and Prepas 1987). Schindler et al. (2008) studied the impact of controlling either nitrogen or phosphorous in order to reduce eutrophication and concluded that in order to reduce eutrophication the focus of eutrophication studies must be on decreasing the phosphorous inputs. The analyses of Downing et al. (2001) concluded that the relationship that is of outmost importance is based on total phosphorous, which predicts phytoplankton biomass, as it is an indicator of the potential for cyanobacterial bloom development, but is not a good indicator of cyanobacterial dominance, as other factors also play an important role, e.g. temperature and light. They suggested that the risk profile for cyanobacteria dominance is 0-10% between 0 and 30 ug/L total P, rising to 40% between 30 and 70ug/L and reaching asymptote at 80% near 100ug/L. Carpenter (2008) stated it differently in that even though the focus should be on phosphorous control, nitrogen enrichment should not be ignored.

2.7 *Microcystis*

Blooms of *Microcystis* develop in standing and slow-moving freshwaters all around the world as well as in marine coastal waters (Parra et al. 1980). Different sized lakes from small ponds to large lakes can have *Microcystis* and common amongst these is the stability of their water column for some part of the year (Visser et al. 2005). *Microcystis* blooms during eutrophication can cause problems such as odours, off-flavour compounds and various toxic substances which may have negative impacts on the drinking water quality and the overall treatment costs (Falconer 2005). *Microcystis* blooms are the most important cyanobacterial bloom forming species in the northern parts of South Africa (Van Ginkel 2008).

2.8 Light

Light plays a major role in bloom development. In the study by Reynolds et al. (1981) it was suggested that the occurrence of *Microcystis* blooms is affected by the interaction between light attenuation and stability of water column together with high nutrient loadings, pH, CO_2 and low redox potential. Increasing the light intensity leads to photosynthetic activity reaching a maximum while further increases lead to declining activity (Yagi et al. 1994).

2.9 Wind

Mixing induced by wind in the lake result in horizontal heterogeneity in the distribution of the bouyant phytoplankton population in the lake (Webster 1990), as it causes movement within the epilimnion during summer periods, especially if the stratification of the system is stable. During stratification, surface water (epilimnion) shows higher light intensity, temperature and dissolved oxygen concentrations in comparison to deep waters (hypolimnion) (Yu et al. 2014). The bottom water is nutrient rich with very little growth taking place due to light limitations. During mixing after stratification the nutrients, sulphides, and other dissolved constituents are conveyed to the surface waters, potentially triggering eutrophication symptoms and its problems (Lawson and Anderson 2007).

2.10 Temperature

Temperature is one of the key attributes for cyanobacteria and *Microcystis* development in particular throughout the year (Reynolds et al. 1981). It is critical in the regulation of buoyancy which affects gas vesicle synthesis, rate of photosynthesis and carbohydrates production (John et al. 2008).

2.11 Dissolved Oxygen

In one of the detailed ecological studies of *Microcystis* it was concluded that the viability and recruitment of colonies is supported by anoxic conditions. Recruitment of colonies is supported by anoxic conditions in the hypolimnion (Guseva 1952). Some of the *Microcystis spp.* are said to be more tolerant to anoxic conditions which are the most likely conditions in the eutrophic system beneath thick surface scums in the water column and bottom sediments. This ability allows *Microcystis* to dominate in eutrophic lakes (Shi et al. 2007).

2.12 Sources of nutrients in water

The sources of nutrients in water are said to vary from agriculture, aquaculture, septic tanks, urban wastewater, urban storm water runoffs, industry and fossil fuel combustion (WRI 2009). In the survey of the global nutrient sources it was noted that in the United States and the European Union, agricultural sources (i.e. commercial fertilizers and animal manure) are typically the primary sources of nutrient impairment in waterways, while urban wastewater is the primary source in Asia and Africa (WRI 2009).

Table 2.1 Primary Sources and Pathways of Nutrients (redrawn from WRI 2009)

	Pathways		
Sources	Air	Surface Water	Ground water
Sewage Treatment plants		✓	
Industry	✓	✓	
Septic systems		✓	✓
Urban stormwater runoffs		✓	
Agricultural fertilisers	✓	✓	✓
Livestock operations	✓	✓	✓
Aquaculture		✓	
Fossil fuel combustion	✓		

The main source of phosphorous for *Microcystis* species is orthophosphates and additionally, they can use organic forms of phosphorous. They can source nitrogen as NO_3, NO_2 or NH_4 but not as N_2 because of the absence of nitrogenase (Whitton 2012).

The key drivers of eutrophication may be classified into two categories i.e. direct and indirect sources. Indirect drivers include population growth; economic growth in the developing world, which will impact consumer consumption; and the growth of intensive agriculture. Direct drivers of eutrophication include higher energy consumption, increased fertilizer consumption, and land-use change.

2.12.1 Urban and Industrial sources

The municipal sources include discharges from waste water treatment plants, industrial waste water discharges, nitrogen leaching from underground septic tanks, and storm water runoff. These are considered point sources of nutrient pollution because they discharge directly to surface or ground water through a pipe or other discrete conveyance (WRI 2009). These sources are relatively easy to control and are often regulated in most of the developed countries. In most countries sewage is the most significant contributor due to the growing population numbers which are often associated with urbanization and increased pressure on water resources. Sewage sometimes combines with storm water runoff and these may result in volumes that are in excess of the design capacities of the wastewater treatment plants receiving the flow. This results in excess nutrient rich water, including raw sewage, being discharged directly into the nearby streams and rivers.

In Sub-Saharan Africa, it is said that the population in major cities is increasing rapidly and only about 30% of the produced sewage is treated in sewage treatment plants and that 60% is disposed of untreated (via onsite treatment systems) and the balance remains unaccounted for (Nyenje 2010).

2.12.2 Agricultural Sources

Fertilizer leaching, runoff from agricultural fields and manure from concentrated operations and aquaculture are the largest agricultural nutrient sources (WRI 2009). The use of fertilizers with high concentrations of nitrogen and phosphorous have increased, over the last century. This has been necessary to keep up with the ever growing population. The negative that came out of this is that about 20% of the applied fertilizers are lost through volatilization, surface runoff and leaching to groundwater (MA 2005). The nitrogen in synthetic fertilizers and manure in the field is also subject to volatilization. Nitrogen in the form of ammonia is lost through volatilization and losses may be as high as 40% (MA 2005). It is still discharged to the water resources eventually through precipitation.

2.12.3 Fossil Fuel Sources

The burning of fossil fuels releases nitrogen oxides into the atmosphere. These gases lead to the formation of smog and acid rain. In the Witwatersrand drainage area several incidences of acid rain coming from pyrite contamination associated with coal and gold mining have been reported (McCarthy 2011). The challenges of mining and associated Acid Mine Drainage (AMD) problems are well documented.

These are the main source of acidic water within the South African context. The sulphur dioxide and nitrogen oxides, when dissolved in water, ultimately form strong mineral acids, namely sulphuric and nitric acid. These are redistributed to land and water through rain and snow. These acids in the catchments leach calcium, magnesium and phosphorous from the soil, as well as increase the concentration of inorganic nitrogen (Grafton RQ and Hussey K 2011). In South Africa, the Highveld region which stretches across Gauteng, Free State and Mpumalanga, accounts for about 90% of the nitrogen oxide that is emitted in South Africa (Collett et al. 2010). Around this region several incidences of acidic rainwater have been recorded with pH as low as 4.72 in the Kruger National Park (Mphepya et al. 2006). In the United States' Northern Atlantic, atmospheric deposition of nitrogen can exceed riverine nitrogen inputs to coastal areas (Spokes and Jickells 2005).

The main drivers of eutrophication may be categorized into direct and indirect drivers. The indirect drivers include population growth, economic growth and growth of intensive agriculture. The increasing population density increases the demand for food, land, energy and other natural resources. The consequence of which is greater agricultural production and increased burning of fossil fuels. Economic growth leads to changes in consumption patterns such as the different dietary choices, increased energy use and increased consumption of consumer goods (WRI 2009).

The FAO report projects an increase in the fertilizer consumption (FAO 2000). The main driver is the developed and developing nations where intensive agricultural practices are employed and this result in nutrient pollution, which is the unintended consequence of intensive agriculture (FAO 2000, WRI 2009, Blignaut 2012).

Land use conversion from perennial vegetation to annual cropping has also contributed negatively toward increased eutrophication. The increased crop lands come at an expense of forests and some wetlands. The natural systems such as forests and wetlands play an important role in the capturing and cycling of nutrients. The increased land use conversions result in the reduced ability of the landscapes to intercept nutrients. This leads to greater nutrient loss into local watersheds (WRI 2009).

Cyanobacteria blooms are amongst the many symptoms of eutrophication. There has been an increasing trend in the amount of cyanobacterial blooms recorded

(Codd et al. 2005, Mathews & Bernard 2015). The increased incidence may be attributed to increasing eutrophication and increasing water temperatures (Van Ginkel & Silberbauer 2007). For South Africa's hypertrophic reservoirs a CEGAP model has been developed as a tool to predict the emergence of cyanobacterial blooms (Van Ginkel 2008).

2.13 Eutrophication Monitoring in South Africa

Eutrophication monitoring in South Africa commenced in early 1970's where it was being done as ad hoc monitoring surveys and research projects supported by Water Research commission (WRC) up to about 1985 (Toerien et al., 1975). One of the other early initiatives of eutrophication monitoring was the Trophic Status Project (TSP) which was commissioned by the Department of Water and Forestry for 49 of the 250 South African Impoundments at the time (Van Ginkel et al., 2000). The objectives of the TSP were the following:

- To determine time-related project changes during the monitoring programme.
- To investigate trends in nutrient concentrations and eutrophication symptoms in selected South African impoundments.
- To determine the trophic status of impoundments in the catchments described as being sensitive.
- To evaluate the effect of the 1mg/L, P effluent discharge standard (P Standard) on the trophic status of selected impoundments.
- Provide Water Resource Managers (National and Regional) with information that can be used in the development of future eutrophication monitoring programmes and management strategies.

The project laid a solid foundation for the current National Eutrophication Monitoring Programme (NEMP), in that it highlighted the extent of the problem at a national scale and provided a database that was to be used for the design of the NEMP (DWAF, 2002). The variables such as chlorophyll-*a*, total phosphorous, the percentage of cyanobacteria and Secchi disc depth were being measured for TSP and these formed the NEMP database.

The National Eutrophication Monitoring Programme (NEMP) is the water quality monitoring program used by Department of Water and Sanitation to monitor the eutrophication status and potential for reservoirs, lakes and rivers in South Africa. The main objective of NEMP is defined in the implementation manual as to measure, assess and report regularly on:

- trophic status, the nature of eutrophication problems, the potential for future changes in trophic status in South African impoundments and rivers in a manner that supports strategic decisions.

In the manual the following criteria is used to classify the dams and lakes regarding the extent of nutrient enrichment (trophic Status):

Table 2.2 Trophic status as used for assessment of reservoirs in South Africa

Oligotrophic	Low in nutrients & not productive in terms of aquatic animal & plant life
Mesotrophic	Intermediate levels of nutrients, fairly productive & showing emerging signs of water quality problems
Eutrophic	Rich in nutrients, very productive & showing increasing signs of water quality problems
Hypertrophic	Very high nutrient levels where plant growth is determine by physical factors. Serious water quality problems

Increased nutrient levels can stimulate other forms of primary production, in addition to algae and cyanobacteria. Many nutrient-enriched water bodies are often choked with excessive growths of aquatic macrophytes, which can influence recreational activities (especially swimming & boating) and alter the structure of the food web that can have an economic impact (limiting fishing).

Table 2.3 Method to determine trophic status statistics

Statistic	Unit	Current trophic status			
chlorophyll *a* (median)	µg/l	0<x≤10	10<x≤20	20<x≤30	>30
		Oligotrophic (low)	Mesotrophic (Moderate)	Eutrophic (significant)	Hypertrophic (serious)
% of time chl *a*> 30µg/*l*	%	0	0<x≤8	8<x≤50	>50
		negligible	moderate	significant	serious
Potential for algal and plant productivity					
TP (Median)	mg/l	x≤0.015	0.015<x≤0.047	0.047<x≤0.13	>0.13
		negligible	moderate	significant	serious

The determinants that are measured as part of the National Eutrophication Monitoring Programme include suspended solids, chlorophyll *a*, algae species, macro chemical variables such as suspended solids (SS), ammonium (NH4), nitrate and nitrite (NO3+ NO2), ortho-phosphorus (PO4-P), sulphate (SO4), silica (Si), total alkalinity (TALK), the Kjeldahl nitrogen (KN), total phosphorus (TP) and Secchi disc depth.

CHAPTER 3

STUDY AREA, MATERIALS AND METHODS

3.1 STUDY AREA

The study was conducted for the Klipvoor Dam which is located in North West province of South Africa within the Crocodile West and Marico Water Management Area (WMA). The province is one of the mining hubs of South Africa with mining and agricultural activities as the main economic drivers. Both these activities are very demanding on the water supply and do impact negatively on water quality in the region. In the study by Matthews (2015), Klipvoor Dam was identified as one of the reservoirs with significant increases in cyanobacterial infestation together with Vaalkop Dam. The Klipvoor Dam is located on the Pienaars River downstream of the Bon Accord Dam and the dam wall construction was completed in 1970. The dam was built to regulate the flow of the Pienaars River and to provide assurance of water supply for downstream irrigation. The dam was proposed for provision of domestic water in Ga-Rankuwa, Uitvalgrond, Leboneng, and the South African Police Training Centre in Hammanskraal. The catchment area comprises of agricultural land and rural informal settlements. The reservoir is situated within the Borakalao National Park, a nature reserve that protects the riparian zone of the impoundment. Within the reserve are various animal species such as the threatened white rhino, giraffe, leopard and zebra species. Other uses for the dam include recreational fishing and downstream irrigation. The reservoir is downstream of the Roodeplaat Dam of which the catchment consists to a large extent of urban developments. The most dominant landcover was woodlands/open bush (48%), followed by urban area (14%), grass lands (12%), then cultivated lands (11%), thicket (6%) and wetland (2%).

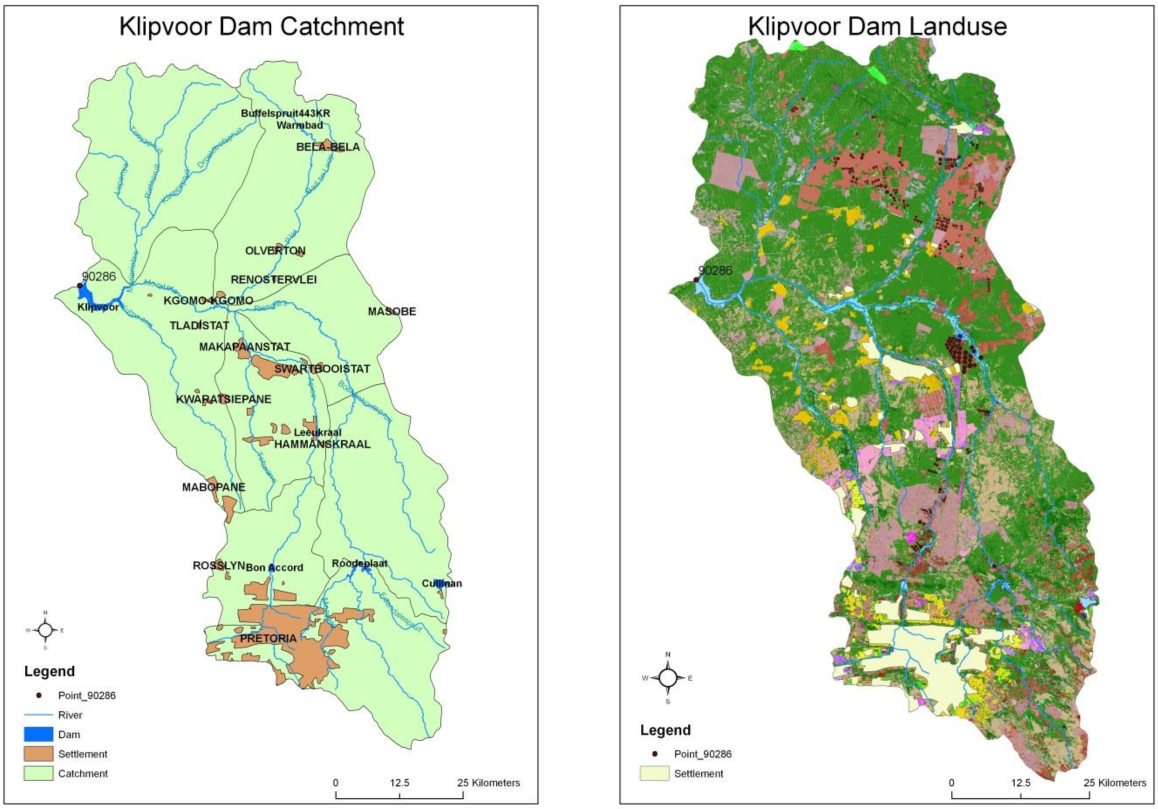

Figure 3.1: Klipvoor Dam landuse and catchment maps

3.2 MATERIALS AND METHODS

3.2.1 Water Quality Monitoring and sampling

Water quality samples were collected by the Department of Water and Sanitation (DWS) samplers as part of the National Eutrophication Monitoring Program (NEMP). The samples are collected fortnightly for analyses at the Resource Quality Information Services laboratories, Roodeplaat Dam, Pretoria.

The samples were collected from the dam wall. These sample sites are used for NEMP as they are positioned to provide a cumulative impact of the pollution coming into the dam. The physical parameters such pH and electrical conductivity are taken *in situ* and in the lab. Other *in situ* measurements include dissolved oxygen and temperature which are measured at every 1-meter depth interval.

The samples were collected with a 5-meter hosepipe for an integrated sample and at 1-meter for 1-meter depth sample. Each sample is emptied into a 10-litre plastic bucket and then subdivided into a macro inorganic sample, an algal identification sample and chlorophyll–*a* sample. The macro inorganic samples are analyzed on automatic analyzers at Resource Quality Information Services (RQS) laboratories of DWS. The variables measured were: Suspended solids (SS), ammonia (NH_4), nitrate and nitrite ($NO_2 + NO_3$), orthophosphorus (PO_4-), sulphate (SO_4), silica (Si), and total alkalinity (TAL). Other determinants such as Total Kjeldahl nitrogen (KN) and total phosphorus were measured using the digestion methods from the same macro inorganic sample. Total nitrogen is the sum of ammonia, nitrate and nitrite concentrations. Phytoplankton samples were collected in a 1-litre plastic bottle and put in a cooler box packed with ice, for analyses. Sample preparation involves pouring a 10ml sample into a counting chamber where it is left to settle overnight or for a minimum of six hours. The identification is done with an inverted light microscope and the results are expressed as a percentage of the total algal population.

The chlorophyll-*a* sample is prepared by filtering 500ml of the sample through a 45µm Whatman filter paper. The chlorophyll-*a* are then extracted into a 10ml ethanol solution for measurement. The chlorophyll-*a* concentrations are measured by the spectrophotometry method of the RQIS laboratories.

The biovolume for algal species was calculated as follows:

AB = (AD/100)*(Chl-*a* * 2.5) (Van Ginkel et al. 2007)

Where :

AB is the algal bio-volume measured as cm^3/m^3

AD is the algal dominance (measured as %)

Chl-*a* is the measured chlorophyll-*a* concentration (measured in µg/*l*)

During this study a 10 year period with data between January 2005 and December 2015 was considered.

3.2.2 Gaps in the data

Where data gaps were apparent the average of the measurements before and after the gap were used to calculate the average which was an estimate for the value of the gap. Where more than one, consecutive data sets were missing a program was developed using a linear regression techniques. The equation and graph that was plotted and used to fill in the gaps, is presented as figure 4.1 in the next chapter.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 CEGAP Model Evaluation

A total of 10 year biweekly data for Klipvoor Dam was used to test the applicability of the CEGAP model to predict the *Microcystis spp*. blooms for the dam. There are various methods of evaluating the model which include both quantitative and qualitative methods (Bennet et al. 2013). For this study quantitative methods have been used to compare the predicted values of *Microcystis* biovolume and the observed *Microcystis* biovolume. The time series plots of the predicted values of *Microcystis* biovolume and observed *Microcystis* values are compared as well.

The plot of Total phosphorous (TP) versus ortho-phoshorous (PO_4) concentration was used to calculate missing TP values in the data set. The straight line plot had an R^2 value of 0.6785 which was high enough to assume a statistically significant relationship between these two variables in the Klipvoor Dam. A straight line equation of y=0.6918x + 0.0532 was used to calculate the missing values in the data.

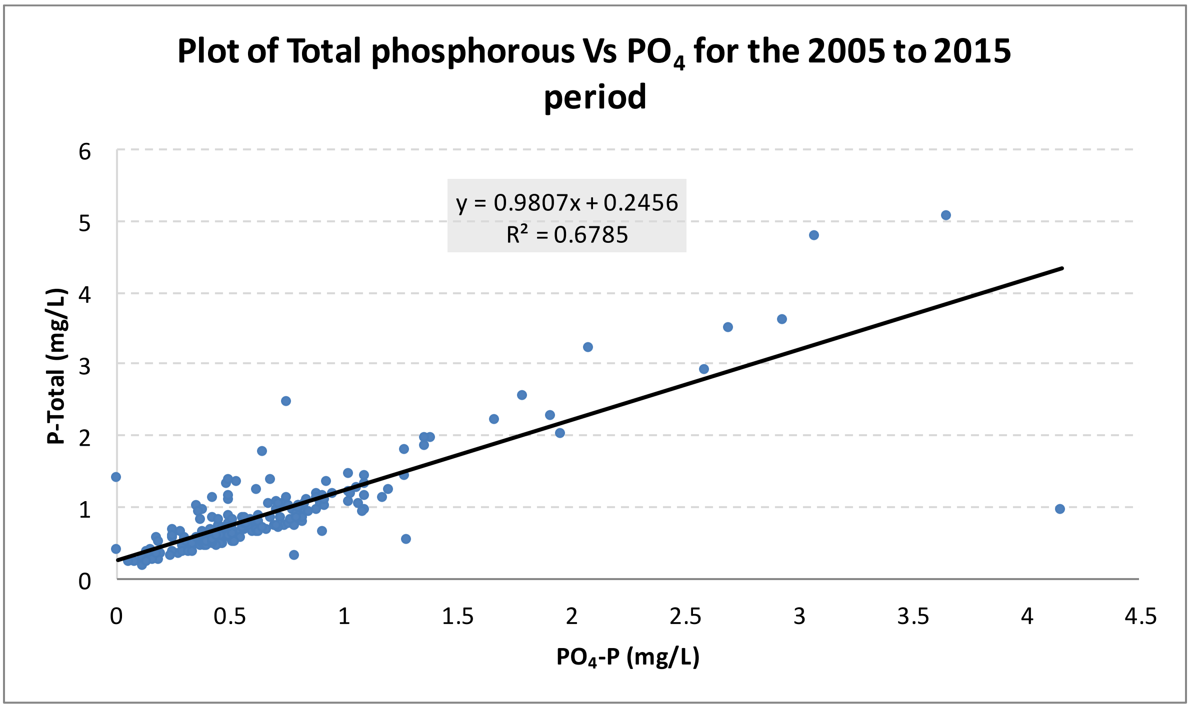

Figure 4.1 A total phosphorous versus ortho-phoshorous plot for the Klipvoor Dam for calculation of missing TP values

4.2 t-test analyses

The t-test is used to assess the statistical significant difference between the observed and predicted values. The test is based on the null hypothesis (H_0) that states that there is no significant difference between the observed values and the values predicted by the CEGAP model. The hypothesis is rejected where the p-value from the t-test is less than 0.05. Below are the t-test results for the real time, 14-day and 28-day predictions.

Table 4.1 t-test results for the real-time, 14-day and 28-day predictions

CGAP Prediction	**Real time**	**14-day**	**28-day**
p-value	0.008866	0.007417	0.007417

In all of the above instances the p-values is less than 0.05, this means that we have to reject the null hypothesis and adopt the alternative hypothesis which states that there is a statistically significant difference between the observed values and the predicted values.

4.3 Direct Comparison

This is a method that is used to assess and compare the similarities between the predicted and observed values.

Table 4.2 The descriptive statistics for observed/real time (RT) values and Real time forecasted (RTF), 14 and 28 days forward values of *Microcystis* biovolume for Klipvoor Dam.

Variable	***Valid N***	***Mean***	***Median***	***Min***	***Max***	***Std.Dev***
Mic RT	252	309.01	0	0	19491.2	1398.8
Mic RTF	253	33.4	11.4	0	2848.5	185.7
Mic 14	252	30.0	13.1	2.1	866.1	67.6
Mic 28	252	10.8	11.9	0	29.4	5.67

The analysis shows that the predicted values are not in the same order as the observed biovolume values. The average predicted values for *Microcystis* biovolumes in the Klipvoor Dam are undervalued by the model. The high values could be due to high levels of biomass during the time of the analyses caused by the algae blooms which may not necessarily be related to *Microcystis*. There were several instances where the concentration of *Ceratium* was dominant and the biomass in these instances was high, even in the absence of the *Microcystis* species. These high biomass values have the potential to affect the accuracy of the model, which only relies on the *Microcystis* component of the biomass for the predictions. The model is designed to include factors in the component of the biomass that comes from other algal species than the *Microcystis* contribution. The model uses percentage abundances for different algae species and therefor accounts for various algae species. Even though this factor is built into the model there is a high probability that the *Ceratium* contribution to biomass may be the cause for the model discrepancies in terms of the predictions, as higher chlorophyll-*a* values may be recorded and apportioned towards *Microcystis* while in reality they are due to *Ceratium* and this will mean a higher biovolume.

4.4 Visual inspection

A time-series was used to exhibit the observed versus the predicted values for the different forecasts. The plots of the observed values and the predicted values using the CEGAP model for the real time (Figure 4.2), 14-day (Figure 4.3) and 28-day (Figure 4.4) predictions are presented in the graph below:

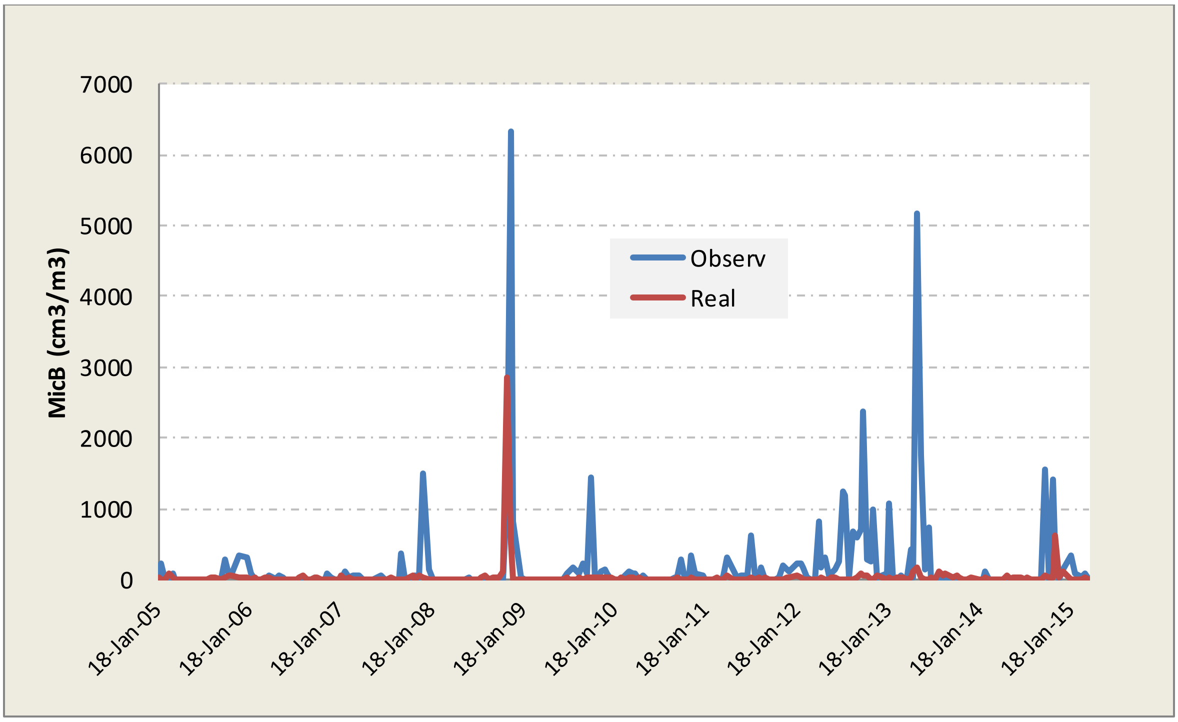

Figure 4.2 Predicting the abundance of *Microcystis sp*. in the Klipvoor Dam for period 2005 to 2015. Observed (blue) vs. Real time predicted (red) time-series values for real time (RT).

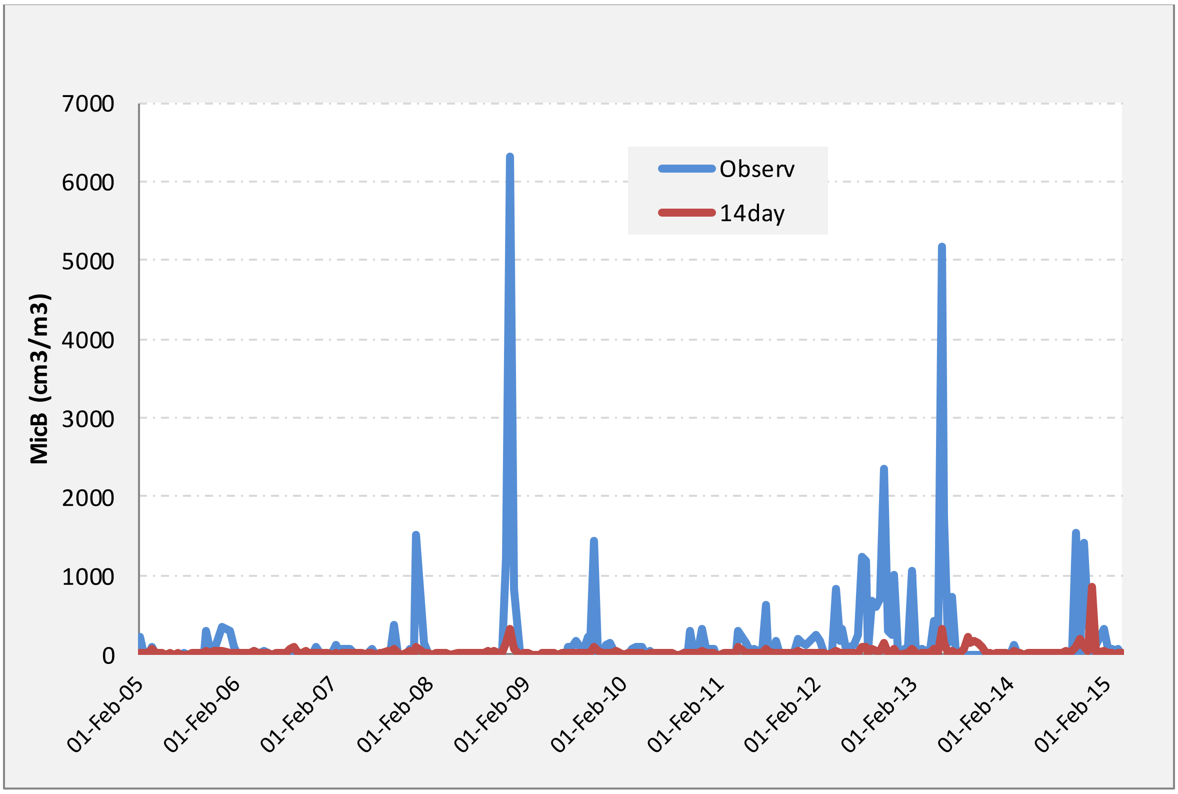

Figure 4.3 Predicting the abundance of *Microcystis sp.* in the Klipvoor Dam for the 2005 to 2015 period. Observed (blue) vs. predicted (red) time-series values for 14-days forward.

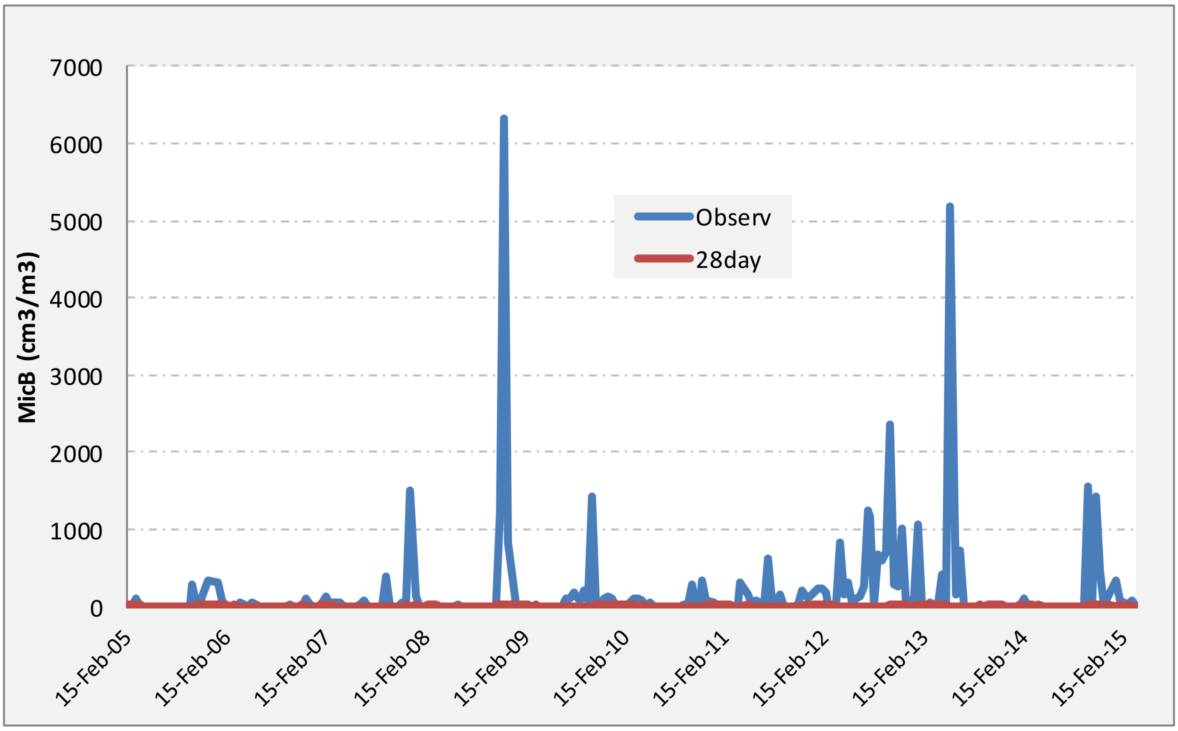

Figure 4.4 Predicting the abundance of *Microcystis sp.* in the Klipvoor Dam for the 2005 to 2015 period. Observed (blue) vs. predicted (red) time-series values for 28 days forward.

The graphs in figure 4.2 – 4.4 above indicate that although there are differences in the ranges of the predicted and observed values, the model is able to predict the presence of peaks. The significant similarities were observed between the observed data and the temporal prediction of an upcoming *Microcystis* bloom for the real time and 14-days forward predictions. There were no significant acceptable forward prediction results as compared to the observed values of *Microcystis* blooms and the 28-day forward prediction.

The time series plots indicate a strong relationship between the observed and the predicted values for the real time and 14-day predictions. The peaks indicative of the algal bloom events are seen to be occurring around the same times even though they are at different intensities. This indicates that the model predicts the occurrence of *Microcystis* blooms even though it is not accurate on predicting the severity of the event. The discrepancies in severity may be attributed to the large cells of *Ceratium* species, which have higher chlorophyll-*a* concentration contents and which are prevalent in the Klipvoor Dam. It has the potential to increase the overall biomass but will not account for the *Microcystis* contribution. Downing et al. (2001) arrived at a similar observation where it was stated that the relative contribution of Cyanobacteria to total phytoplankton biomass follows a non-linear relationship with phytoplankton biomass, N:P stoichiometry and nutrient concentrations. The increase in the phytoplankton biomass may be predicted from the total phosphorous concentrations. In instances of high *Ceratium* numbers the models is likely to have discrepancies in predicting severity of the *Microcystis* biomass since the biomass count can be adversely affected by the larger *Ceratium* cells and other algal species which are not necessarily accounted for in the model.

4.5 Correlation Coefficient Analyses

A correlation is the measure of association between two variables. The correlation may either be a positive correlation where a high value on one variable is associated with the high value on another; or negative correlation where the low variable is associated with a high on another variable.

The data analyses show a strong positive Pearson's correlation value of 0.9262517, this value indicates a strong positive relationship between the observed values and the 14-day predicted values. This strong relationship imply that when the values of the observed values increase the 14-day prediction follows similar trends and when they decrease to lower values a similar trend is followed by the predicted values.

For the 28-day prediction a weak positive Pearson's correlation coefficient value of **0.06655496** was calculated. This also indicates that the observed values do increase in a similar manner as the real values, but there is no significant correlation.

The comparison of the real-time predicted value versus the observed value had a weak positive Pearson's correlation value of **0.1102023**. This low value indicates a weak correlation between the observed data and the real-time predicted data. There is therefore no significant correlation between the observed and real time predicted values.

4.6 Prediction of *Microcystis* blooms using CEGAP Model

The 10-year bi-weekly data was used to assess the applicability of the CEGAP model in predicting *Microcystis* sp. blooms in the Klipvoor Dam. The observed *Microcystis* biovolume values are calculated by multiplying the chlorophyll-*a* values with the *Microcystis* abundance. The CEGAP model calculation in this study only considered the biomass resulting from the *Microcystis* species at a given time. The exclusion of all other algal species from the prediction means that where algal blooms are not due to *Microcystis* the model will not be able to predict the period and severity at which they may emerge.

It was noticed that in Klipvoor Dam there are various algal species which at times dominate the dam and in the process contribute differently in the chlorophyll-*a* counts. In instances where the *Microcystis* in 100% dominant it stands to reason that the chlorophyll-*a* figures and the estimations of the biomass will be more accurate.

The predicted values are calculated using the fortnightly values of chlorophyll-*a*, and *Microcystis* abundance. The challenge with these predictions is that it turns out to be vulnerable to inflated chlorophyll-*a* concentrations which may be due to other algal species other than the *Microcystis* species. In the algae data for Klipvoor Dam there were several instances where *Ceratium* species were the most dominant algae species for the dam and this means that there will be instances where the high chlorophyll-*a* numbers will be directly due to high *Ceratium* numbers.

The model was, according to visual inspections, more accurate in predicting the trend of emergence of the *Microcystis* blooms in the dam. The model modeled the emergence of the real-time and the 14-day forward predictions. There was very little correlation between the 28-day forward prediction and the real data.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

The CEGAP model was used to predict the emergence of the *Microcystis* blooms in the Klipvoor Dam, the model was able to make predictions with reasonable accuracy for the emergence of blooms for real time and 14-day forward predictions. However the 28-day forward predictions were not reliable. In all instances the model was able to predict emergence of blooms but was not able to accurately predict the severity of the blooms. The predictions for the real time and the 28-day predictions were not accurate for the CEGAP model.

The larger size of especially Ceratium as compared to the *Microcystis* species, and the potential thereoff to contribute a larger percentage of the biomass and the chlorophyll-*a* concentration, even though they may have less abundance in terms of species counts, ma account for the discrepancies. The presence of the *Ceratium* species which are larger and provide additional biomass was not accounted for in the model.

These results indicate that the model is able to predict the emergence of the *Microcystis* biomass, but the severity of the biomass may be influenced by other algae species.

Recommendations:

- The model should be developed further in order to be able to predict the severity of all potentially dominant algal blooms.
- The model should be tested or adapted to enable the prediction of other dominant algal species, especially *Ceratium* specie, which are often dominant in the Klipvoor Dam.
- The CEGAP model should be tested for other regions of the country to assess its capability in predicting bloom events in different climatic zones of the country e.g. coastal areas such as KwaZulu-Natal and Western Cape.
- The model should be test for use by DWS to alert water sports associations about the algae bloom events during planning stages of these sports.

- There is a need to updated the model and incorporate some climate change variables in order to be able to predict future blooms.

REFERENCES

Art HW (1993). A dictionary of ecology and environmental science (1st ed.): New York, Henry Holt and Company, pp. 196.

Barel CDN, Dorit R, Greenwood PH, Fryer G, Hughes N, Jackson PBN, et al. (1985). Destruction of fisheries in Africa's lakes. *Nature*, **315(6014):**19–20.

Barnard S, van Ginkel CE, Coetzee LZ and Booyens S (2014). Implementation of the rule based agent for *Microcystis* in Rietvlei Dam, *WRC Report* **No. 19662/1/13**. Water Research Commission, Pretoria.

Berman T (1997). Dissolved organic nitrogen utilisation by *Aphanizomenon* bloom in Lake Kinneret. *J Plankton Res*, **19**:577-586

Blignaut J and De Wit M (2012). Sustainable Options: Economic development lessons from applied environmental resource economics in South Africa (reprint) Cape Town, South Africa, UCT Press.

CENR (2000). Committee on Environment and Natural Resources (2000) Integrated assessment of hypoxia in the Northern Gulf of Mexico: *National Science and Technology Council*, 58 p.

Chorus, I (2001). Cyanotoxins: occurrence, causes, consequences. Springer. Berlin, New York, pp. 357.

Clark BM (2006). Climate change: A looming challenge for fisheries management in southern Africa. *Marine Policy*, **30**: 84–95

Codd GA, Azevedo SMFO, Bagchi SN, Burch MD, Carmichael WW, Harding WR, Kaya K and Utkil HC (2005). CYNONET A global network for cyanobacterial bloom and toxin risk management. IHP-IV. *Technical Documents in Hydrology*, **76**. UNESCO. Paris. 138pp.

Collett KS, Piketh SJ and Ross KE (2010). An assessment of the atmospheric nitrogen budget on the South African Highveld. *S Afr J Sci*, **106 (5/6)**, Art. #220, 9 pages.

Department of Water Affairs Forestry (2002). National Eutrophication Monitoring Programme. Implementation Manual. Compiled by K Murray, M du Preez and CE van Ginkel. Pretoria South Africa.

Falconer IR (2005). Cyanobacterial toxins of drinking water supplies – cylindrospermopsins and microcystins. CRC Press, New York, pp:296.

FAO (2000). The state of Food and Agriculture: Lessons from the past 50 years. Food and Agriculture Organization of The United Nations, Rome.

Figueredo CC, Giani A (2001). Seasonal variation in the diversity and species richness of phytoplankton in a tropical eutrophic reservoir. *Hydrobiologia*, **445(1–3)**:165–74.

Grafton RQ and Hussey K (2011). Water Resource Planning. Cambridge University Press, United Kingdom. 75pp.

Harding B (2008). Microscope refocused on S.A. water quality threat of eutrophication. *Water Wheel,* ***7*****(5)**, 14-17.

Hecky RE, Bugenyi FWB (1992). Hydrology and chemistry of the African Great Lakes and water issues: problems and solutions. *Mitt Int Ver Limnol*, **23**:45–54.

Hitzfeld BC, Hoger SJ and Dietrich DR (2000). Cyanobacterial toxins: Removal during drinking water treatment and human risk assessment. *Environmental Health Perspectives Supplements*, **108**: 1-17

Horne AJ and Goldman CR (1994). Limnology. McGraw-Hill, New York. 480pp.

Graham JL, Loftin KA, Ziegler AC & Meyer MT (2008). Guidelines for design and sampling for cyanobacterial toxin and taste-and odor studies in lakes and reservoirs. *U.S. Geological Survey Scientific Investigations,* **No. 5038**, 39pp.

Graham M, Blignaut M, De Villiers L, Mostert D, Sibande X, Gebremedhin S, Harding W, Rossouw N, Freese NS, Ferrer S and Browne M (2012). Development of a generic model to assess the costs associated with eutrophication. *WRC Report* **No. 1568/1/12**. Water Research Commission. Pretoria, South Africa.

Grobler DC (1986). Assessment of the impact of eutrophication on South African impoundments. *Ecol. Modelling*, **31**: 237-247.

Grobler DC (1985). Phosphate budget models for simulating the fate of phosphorous in South African Reservoirs. *Water SA*, **11**, 219-230.

Guven B and Howard A (2006). Modelling the growth and movement of cyanobacteria in river systems. *Science of Total Environment*, **368**, 898-908.

Kling HJ, Mugidde R, Hecky RE (2001). Recent changes in the phytoplankton community of Lake Victoria in response to eutrophication. In: Munawar M, Becky R, editors. The great lakes of the world (GLOW): food-web, health and integrity. *Leiden*, The Netherlands: Backhuys Publishers; p. 47–65.

Kolodny NH, Bauer D, Bryce K, Klucevsek K, Lane A, Medeiros L, Mercer W, Moin S, Park D, Peterson J, Wright J, Yuen C, Wolfson AJ, Allen M (2006). Effect of nitrogen source on cyanophycin synthesis in Synechcystis sp. strain PCC 6308. *J Bacteriol,* **188**:934-940

Kromkamp J (1987). Formation and functional significance of storage products in cyanobacteria. *N Z J Mar Freshw Res,* **21**:457-465

Lawrence E, Jackson ARW, and Jackson JM (1998). Eutrophication, in Longman Dictionary of Environmental Science: London, England, Addison Wesley Longman Limited, p. 144-145.

Lawson R and Anderson MA (2007). Stratification and mixing in Lake Elsinore, California: an assessment of axial flow pumps for improving water quality in a shallow water eutrophic lake. *Water Res*, **41**:4457-4467

MA (2005), Walter Reid *et al*., Millennium Ecosystem Assessment. Washington, DC: Island Press.

Masango MG, Myburgh JG, Labuschagne L, Govender D, Bengis RG and Naicker D (2010). Assessment of Microcyctins bloom toxicity associated with wildlife mortality in the Kruger National Park. *South Africa. Journal of Wildlife Diseases*, **46(1)**:95-102

Masango MG, Matshoga RG, Ferreira DCH and Sandvick M (2012). An assessment of the toxicity of cyanobacteria in the Kruger National Park. *WRC Report* **No. 1850/1/11**. Water Research Commission. Pretoria, South Africa.

Matthews MW (2014). Eutrophication and cyanobacterial blooms in South African inland waters: 10 years of MERIS observations. *Remote Sensing of Environment*, **155**:161-177.

Matthews MW and Bernard S (2015). Eutrophication and cyanobacteria in South Africa's standing water bodies: A view from space. *S Afr J Sci*, **111** (5/6).

McCarthy TS (2011). The impact of acid mine drainage in South Africa. *S Afr J Sci*, **107** (5/6): 712-718.

Mphepya JN, Galy-Lacaux C, Lacaux JP, Held G and Pienaar JJ (2006). Precipitation chemistry and wet deposition in Kruger National Park, South Africa. *J Atmos Chem*, **53**: 169-183.

Muggide R (1993). The increase in phytoplankton primary productivity and biomass in Lake Victoria (Uganda). *Verh Int Ver Limnol*, **25**:846–849.

NAS (1996). National Academy of Sciences, Introduction, summary, and recommendations, in Eutrophication: Causes, consequences, correctives: Washington, D.C., National Academy of Sciences, p. 3-4.

Nixon SW (1995). Coastal marine eutrophication: A definition, social causes, and future concerns. *Ophelia*, **41**, p. 199-219.

Nyenje PM, Foppen JW, Uhlenbrook S, Kulabako R and Muwanga A (2010). Eutrophication and nutrient release in urban areas of sub-Saharan Africa — A review. *Science of the Total Environment,* **408**: 447–455

Oberholster PJ, Botha AM, Cloete TE (2005). An overview of toxic freshwater cyanobacteria in South Africa with special reference to risk, impact and detection by molecular marker tools. *Biokem,* **17**:57–71.

Oberholster PJ & Ashton PJ (2008). State of nation report: An overview of the current status of water quality and eutrophication in South African rivers and reservoirs. *Parliamentary Grant Deliverable*, 15pp.

Oberholster PJ, Botha AM, Ashton PJ (2009). The influence of a toxic cyanobacterial bloom and water hydrology on algal populations and macroinvertebrate abundance in the upper littoral zone of Lake Krugersdrift, South Africa. *Ecotoxicology,* **18 (1):**34–46.

Oberholster PJ, Botha AM & Myburgh JG (2009). Linking climate change and progressive eutrophication to incidents of clustered animal mortalities in different geographical regions of South Africa. *African Journal of Biotechnology,* **8 (21)**: 5825-5832.

O.E.C.D. (1982) *Eutrophication of Waters: Monitoring, Assessment and Control.* O.E.C.D. Paris. pp 154.

Owour K, Okonkwo J, Van Ginkel C & Scott W (2007). Environmental factors affecting the persistence of toxic phytoplankton in the Hartbeespoort Dam. *WRC Report* ***No*** **1401/3/07**. Water Research Commission. Pretoria, South Africa.

Reynolds CS, Jaworski GHM, Cmiech HA, Leedale GF (1981). On the annual cycle of the blue green alga *Microcystis aeruginosa Kutz* emend Elenkin. *Proc R Soc Lond B Biol,* **293 (1068)**:419-477

Riegman R, Mur LC (1984). Regulation of Phosphate uptake kinetics in *Oscillatoria agardhii*. *Arch Microbiol,* **139**:28-32

Rossouw JN (1990). The development of management oriented models for eutrophication control. *WRC Report* **No. 174/1/90**. Water Research Commission. Pretoria, South Africa.

Schwarz R, Forchhammer K (2005). Acclimation of unicellular cyanobacteria to micronutrient deficiency: emergence of a complex network of cellular responses. *Microbiology*, **151**:2503-2514

Shi XL, Kong FX, Yu Y, Yang Z (2007). Survival of *Microcystis aeruginosa* and *Scenedesmus obliquus* under dark anaerobic conditions. *Mar Freshw Res*, **58**:634-639

Spokes LJ and Jickells TD (2005). Is the atmosphere really an important source of reactive nitrogen to coastal waters? *Continental Shelf Research*, **25**: 2022-2035.

Swanepoel A, Du Preez H, Schoeman C, Van Vuuren JS & Sundram A (2008). Condensed laboratory methods for monitoring phytoplankton, including cyanobacteria, in South African freshwaters. *WRC Report* **No TT 323/08**. Water Research Commission. Pretoria, South Africa.

Toerien DF, Hyman KL and Bruwer MJ (1975). A preliminary trophic Status Classification of some South African impoundments. *Water SA*, **1(1)**:15

Trimbee AM, Prepas EE (1987) Evaluation of total phosphorus as a predictor of the relative biomass of blue-green algae with emphasis on Alberta lakes. *Can J Fish Aquat Sci*, **14**:1337-1342.

Turpin DH (1991). Effects of inorganic N availability on algal photosynthesis and carbon metabolism. *J Phycol*, **27**:14-20.

Van Ginkel C, Cao H, Recknagel F & Duplessis S. (2007). Forecasting of dinoflagellate blooms in warm-monomictic hypertrophic reservoirs in South Africa by means of rule-based agents. *Water SA*, **33** (4): 1-8.

Van Ginkel CE, Hohls BC, Belcher A, Vermaak E & Gerber A (2001). Assessment of the Trophic Status Project. Internal Report No.N/0000/00/DEQ/1799. Institute for Water Quality Studies. Department of Water Affairs and Forestry. Pretoria.

Van Ginkel CE and Silberbauer, MJ (2007). Temporal trends in total phosphorous, temperature, oxygen, chlorophyll *a* and phytoplankton populations in Hartbeespoort Dam and Roodeplaat Dam, South Africa. *African Journal of Aquatic Science*, **32**: 63-70.

Van Ginkel CE (2008). Investigating the applicability of ecological informatics modelling techniques for predicting harmful algal blooms in hypertrophic reservoirs of South Africa. Thesis submitted for the degree of Doctor of Philosophy, North-West University, Potchefstroom.

Venter, A and Herold CE (2001). Validation of the Modified MINLAKE Model on Roodeplaat Dam Final Report. *WRC Report* **No 785/1/99**. Water Research Commission. Pretoria, South Africa.

Von Liebig J (1855). Principles of agricultural chemistry with special reference to the late researches made in England. In: Pomeroy, L.R. (Ed.), Cycles of Essential Elements. *Benchmark Papers in Ecology*, **Vol. 1**. Dowden, Hutchinson, and Ross, UK, pp. 11-28.

Wetzel, RG (2003). What is limnology? *ASLO (Advancing the Science of Limnology and Oceanography)*. Retrieved November 12, 2015.

Wetzel, RG (2001). *Limnology: Lake and River Ecosystems*, 3rd ed. Academic Press. ISBN 0-12-744760-1.

Whitten BA (2012). Ecology of Cyanobacteria II: Their Diversity in Space and Time, Springer, New York, United States of America.

Wicks RJ and Thiel PG (1990). Environmental factors affecting the production of peptide toxins in floating scums of the cyanobacterium *Microcystis aeruginosa* in a hypertrophic African reservoir. *Environmental Science and Technology*, **24**(9):1413-1418.

WRI (2009). Annual Report, *World Resources Institute*, Washington DC, United States of America.

Yagi O, Ohkubo N, Tomioka N, Okada M (1994). Effect of irradiance and temperature on photosynthetic activity of the cyanobacterium *Microcystis* spp. *Environ Technol,* **15(4)**:389-394

Yu Z, Yang J, Amalfitano S, Yu X and Liu L (2014). Effects of water stratification and mixing on microbial community structure in a subtropical deep reservoir. *Sci Rep*, **4**:5821